

Page 7, lines 6-12:

Figure 1. Construction of the pLD-TP-Guy's 13 vector and PCR analysis of spectinomycin-resistant tobacco clones transformed with pLD-TP-Guy's 13. Figure 1A. PCR analysis to show integration of the *aadA* gene, using the 3P and 3M primer pair. Figure 1B. PCR analysis to show integration of the H and L immunoglobulin genes, using the 5P and 2M primer pair. Figure 1C. The plasmid vector pLD-TP-Guy's 13 and primer annealing sites. Lane 1, 1 kb ladder; Lane 2, negative control without template; Lane 3, negative control untransformed plant; Lanes 4-6, transformed plants; Lane 7, the plasmid pLD-TP-Guy's 13.

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Figure 2[A]. Construction of the pZS-TP-Guy's 13 vector and PCR analysis of spectinomycin resistant clones transformed with pZS-TP-Guy's 13. Figure 2A. PCR analysis of spectinomycin-resistant tobacco clones using 8P and 8M primer pair. Figure 2B. PCR analysis of spectinomycin-resistant tobacco clones using 7P and 8M primer pair. Figure 2C. The plastic pZS-TP-Guy's 13 and primer annealing sites. Lane 1, 1 kb ladder; Lane 2, negative control without template; Lane 3, negative control untransformed plant; Lane 4, positive control previously characterized pZS-transformed plant; Lane 5, mutant clone; Lanes 6-10, transformed clones; Lane 11, the plasmid pZS-TP-Guy's 13.

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Figures 3A and 3B. Western blot analysis of antibody light chain expression in [*E. coli*] *E. coli* by the tobacco and universal vectors; Figure 3A, Lane 1, molecular weight markers; Lane 2, negative control (insert in the wrong orientation); Lane 3A, XL1-Blue cells transformed with the pZS-TP-Guy's 13 vector; Lane 4A, negative control (untransformed XL1-Blue cells); [Lane] Figure 3B, positive control Human IgA; Lane 4B, XL1-Blue cells transformed with the pLD-TP-Guy's 13 vector. Blots were probed with AP-conjugated goat anti-human kappa antibody.

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Figures 4A and 4B. Western blot analysis of antibody heavy chain expression in *E. coli* by the tobacco vector. Lane 1, molecular weight markers; Lane 2, negative control (insert in the wrong orientation); Lane 3, negative control (untransformed XL1-Blue cells); Lane 4, XL1-Blue cells

transformed with the pZS-TP-Guy's 13 vector. Samples in [blot A] Figure 4A were sonicated, and those in [blot B] Figure 4B were boiled. Blots were probed with Ap-conjugated goat anti-human IgA antibody.

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Figures 5A-5D. Steps in plastid transformation and regeneration of plastid transgenic plants.

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Figures 6A and 6B. Western blot analysis of antibody expression in Tobacco plastids. Lane 1, molecular weight markers; Lanes 2-4, extracts from different transgenic plants; Lanes 5 and 7, blank[,]; Lane 6, negative control extract from an untransformed plant; Lane 8, positive control human IgA. The gels were run under non-reducing conditions. [Blot A]Figure 6A was developed with AP-conjugated goat anti-human kappa antibodies. [Blot B]Figure 6B was developed using AP-conjugated goat anti-human IgA antibodies.

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Figures 8A and 8B. Figure 8A: Southern blot analysis of the clones transformed with the pZS-TP-Guy's 13 vector. Figure 8B, Lane C, control untransformed Petit Havana; Lanes 1-6, transgenic lines.

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Figures 9A and 9B. Figure 9A: Southern blot analysis of the clones transformed with the pLD-Guy's 13 vector. Figure 9B, Lane C, control untransformed Petit Havana; Lanes 1-6, transgenic lines.

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Figures 10A and 10B. Northern blot analysis of light chain transcripts in the transgenic lines transformed with the pZS-TP-Guy's 13 and pLD-TP Guy's 13 vectors. Figure 10A. RNA gel before transfer. Figure 10B. RNA blot probed with radiolabelled light chain DNA probe. Lane 1, RNA ladder; Lane 2, control untransformed Petit Havana; Lanes 3-5, transgenic lines transformed with pZS-TP-Guy's 13; Lanes 6 and 7, transgenic lines transformed with pLD-TP-Guy's 13; Lane 8, post-

transcriptionally silenced nuclear transformant CAR8841; Lane [nine] 9, expressing nuclear transformant CAR517.

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Figures 11A and 11B. Northern Blot analysis of heavy chain transcripts in the transgenic lines transformed with the pZS-TP-Guy's 13 and pLD-TP Guy's 13 vectors. Figure 11A. RNA gel before transfer. Figure 11B. RNA blot probed with radiolabelled heavy chain DNA probe. Lane 1, RNA ladder; Lane 2, control untransformed Petit Havana; Lanes 3-5, transgenic lines transformed with pZS-TP-Guy's 13; Lanes 6 and 7, transgenic lines transformed with pLD-TP-Guy's 13; Lane 8, post-transcriptionally silenced nuclear transformant CAR8841; Lane 9, expressing nuclear transformant CAR517; Lane 10, expressing nuclear transformant CAR532.

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The sequence of the expression cassette between the two *Xba* I sites in pLD-TP-Guy's 13 is shown in ~~Table 1~~ SEQ ID NO. 1. Nucleotides 1-16 comprise linker sequences and a ribosome binding site. Nucleotides 17-1381 comprise a sequence encoding a mouse heavy chain variable/human IgA2m(2) constant hybrid with linker sequences. The native mouse signal peptide has been replaced with methionine (nt 17-19). The heavy chain variable region (nt 20-358) is from the murine monoclonal Guy's 13 (Smith and Lehner, 1989; U.S. Patent Nos. 5,518,721 and 5,352,446, herein incorporated by reference). The sequence of the human IgA2m(2) constant region (nt 359-1381) has been previously published (Chintalacharuvu ~~et al~~ et al., 1994). Nucleotides 1382-1408 comprise stop codon, linker sequences and a ribosome binding site. Nucleotides 1409-2050 comprise a sequence encoding a mouse light chain variable/human kappa constant hybrid with linker sequences. The native mouse signal peptide has been replaced with methionine (nt 1409-1411). The light chain variable region (nt 1412-1731) is from the murine monoclonal Guy's 13 (Smith and Lehner; U.S. Patent Nos. 5,518,721 and 5,352,446). The sequence of the human kappa constant region (nt 1732-2050) has been previously published (Hieter *et al.*, 1980).

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5[']AAATCTAGAGGAGGGATTTATGCAGACATCTGTGTCCCCCTCAAAAGTC-3[']

SEQ ID NO. 3 and

5[']'-CATACCGGGGACTAGTCACATTCACGGTCACCTCGCG-3[']' SEQ ID NO. 4

The resulting PCR product incorporates a ribosome-binding site utilized by the plastid protein translation machinery and a methionine codon upstream of the first amino acid of ICAM-1. The PCR product is cut with Xba I and Spe I (underlined sequences) and cloned into a vector containing the human IgA2m(2) heavy chain constant region. The resulting chimeric gene encodes one continuous protein consisting of 5 domains of ICAM-1 and the constant region of IgA2m(2). The mature protein produced from this construct starts with the sequence of Met-Gln-Thr-Ser-Val- (SEQ ID NO. 5), and ends with the sequence -Lys-Asp-Glu-Leu (SEQ ID NO. 6). It is predicted to have 800 amino acids and a molecular weight of approximately 80,000. The sequence of the ICAM gene has been published (Staunton ~~et al~~ *et al.* 1988), and is incorporated herein by reference. The entire coding sequence of the chimeric gene is cut out with Xba I and cloned into the pLD vector. The resulting expression vector is used to transform tobacco plastids. The chimeric ICAM-1/IgA protein is expressed in transgenic plastids, and assembles into dimers. This multimeric protein comprises an immunoglobulin heavy chain fused to a functional ligand (ICAM-1 domains 1-5), and binds to a site on human rhinoviruses. It is used in a therapeutic manner to prevent rhinovirus colds.